

## Additional protein intake limits weight regain after weight loss in humans

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Since long-term weight maintenance (WM) is a major problem, interventions to improve WM are needed. The aim of the study was to investigate whether the addition of protein to the diet might limit weight regain after a weight loss of 5–10% in overweight subjects. In a randomised parallel study design, 113 overweight subjects (BMI 29.3 (SD 2.5) kg/m<sup>2</sup>; age 45.1 (SD 10.4) years) followed a very-low-energy diet for 4 weeks, after which there was a 6-month period of WM. During WM, subjects were randomised into either a protein group or a control group. The protein group received 30 g/d protein in addition to their own usual diet. During the very-low-energy diet, no differences were observed between the groups. During WM, the protein group showed a higher protein intake (18% v. 15%;  $P < 0.05$ ), a lower weight regain (0.8 v. 3.0 kg;  $P < 0.05$ ), a decreased waist circumference (−1.2 (SD 0.7) v. 0.5 (SD 0.5) cm;  $P < 0.05$ ) and a smaller increase in respiratory quotient (0.03 (SD 0.01) v. 0.07 0.01; (SD)/ $P < 0.05$ ) compared with the control group. Weight regain in the protein group consisted of only fat-free mass, whereas the control group gained fat mass as well. Satiety in the fasted state before breakfast increased significantly more in the protein group than in the control group. After 6 months follow-up, body weight showed a significant group × time interaction. A protein intake of 18% compared with 15% resulted in improved WM in overweight subjects after a weight loss of 7.5%. This improved WM implied several factors, i.e. improved body composition, fat distribution, substrate oxidation and satiety.

### Substrate oxidation: Weight maintenance: Body composition: High protein

A common treatment for obesity is weight reduction. Although short-term weight loss programmes have proved to be successful, long-term weight maintenance (WM) is a major problem (Kramer *et al.* 1989; Wadden *et al.* 1994; Pasman *et al.* 1997a,b; Westerterp-Plantenga *et al.* 1998). Successful weight maintenance is of importance for lowering risk factors for cardiovascular and other diseases (Goldstein, 1992; Wing *et al.* 1992; Van Gaal *et al.* 1997). To improve the metabolic profile, it is not necessary to achieve the ideal body weight: a weight reduction of 5–10% is often sufficient to induce a clinically relevant effect (Goldstein, 1992). To preserve these beneficial effects of weight reduction, an improvement in long-term WM is necessary.

Pasman *et al.* (1999) showed that weight regain was slower when the body composition of the weight regained consisted of a greater fat-free mass (FFM) due to physical activity. We hypothesise that weight regain may be limited if the inevitable increase in body weight consists of a larger FFM, for instance achieved by the consumption of an appropriate substrate. For this, we suggest an increased protein intake, because of its potential to increase FFM (Jean *et al.* 2001). Furthermore, it is known that, of all the macronutrients, protein seems to be the most satiating. Protein consumption suppresses short-term food intake more than that of fats or carbohydrates, and even more

than is expected from its energy content alone (Anderson 1994). Several studies have shown that a high-protein lunch decreases later food intake more than a lunch low in protein (Booth *et al.* 1970; Barkeling *et al.* 1990; Latner & Schwartz, 1999). Westerterp-Plantenga *et al.* (1999) showed an increased satiety effect of a high-protein diet despite similar energy intake. Finally, protein has also been shown to have low energy efficiency during overfeeding (Dulloo & Jacquet, 1999; Stock, 1999), a situation that is comparable to a weight regain situation. Although the effect of dietary protein has been examined in weight-loss studies (Skov *et al.* 1999; Dumesnil *et al.* 2001; Layman *et al.* 2003), the effect of additional protein intake on WM has not yet been studied. The aim of this study was to investigate whether the addition of protein to the diet might limit weight regain after a weight loss of 5–10% in moderately overweight subjects.

### Subjects and methods

#### Subjects

One hundred and forty moderately overweight male and female subjects (BMI 25–35 kg/m<sup>2</sup>, age 18–60 years) were recruited for this study by advertisements in local

**Abbreviations:** FFM, fat-free mass; FM, fat mass; PAL, physical activity level; REE, resting energy expenditure; RQ, respiratory quotient; TBW, total body water; TEE, total energy expenditure; TFEQ, Three Factor Eating Questionnaire; VLED, very-low-energy diet; WM, weight maintenance.

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newspapers. They underwent a medical screening. All the subjects were in good health, were non-smokers, were not using medication and were at most moderate alcohol users. A written informed consent was obtained from all the participants. The Medical Ethics Committee of the Academic Hospital in Maastricht approved the study. Twenty subjects did not start the study due to relocation, a change of job or an inability to fulfil the schedule with visits to the university. Intention-to-treat applied for 120 subjects. During the first week, seven subjects dropped out because of difficulty maintaining the very-low-energy diet (VLED). They had been meant to participate in the additional-protein group. Their baseline characteristics did not affect the averages of the baseline measurements of the treatment group. Thus, 113 subjects completed the study. Subject characteristics are shown in Table 1.

### Experimental design

After the subjects' baseline characteristics had been determined, they were divided into two similar groups, stratified for gender, BMI, age, eating behaviour (Three Factor Eating Questionnaire (TFEQ); Stunkard & Messick, 1985; Westtererp-Plantenga *et al.* 1999) and resting energy expenditure (REE). Both groups followed a VLED intervention for 4 weeks in order to initiate weight loss. After this weight loss period, a WM period of 6 months followed. Subjects in both groups visited the university six times. During these visits, the measurements were carried out as described in the Measurements section below. Moreover, the subjects were asked by a dietitian how they felt, how they considered taking part in the study and whether they had any specific questions. Furthermore, dietary counselling was provided upon request for all subjects.

One of the groups was provided with additional protein (protein group, *n* 53), while the other group did not receive this additional protein (control group, *n* 60). Since, similar to previous meal-replacement studies (Ditschuneit *et al.* 1999; Flechtner-Mors *et al.* 2000; Rothacker 2000; Ashley *et al.* 2001; Allison *et al.* 2003), no placebo was used for this control group, we included repeated measurements of dietary restraint to check whether additional dietary protein would affect attitudes towards eating differently. After 6 months of WM, the additional protein intake was stopped. Six months after WM, all the subjects were asked to return to the university for a follow-up measurement of body weight. For this measurement, thirty-one subjects in the protein group and thirty-nine subjects in the control group returned.

### Measurements

The following measurements were executed to determine subject characteristics (Table 1).

**Body weight and BMI.** Body weight was measured on a digital balance (model 707, Seca, Hamburg, Germany; weighing accuracy of 0.1 kg) with subjects in their underwear, in a fasted state and after voiding their bladder. Height was measured using a wall-mounted stadiometer (model 220, Seca, Hamburg, Germany).

BMI was calculated as body weight divided by height<sup>2</sup> (kg/m<sup>2</sup>).

**Waist:hip ratio.** The distribution of fat was investigated by measuring the waist and hip circumferences, and then calculating the waist:hip ratio. The waist circumference was measured at the site of the smallest circumference between the rib cage and the iliac crest, with the subjects standing. The hip circumference was measured at the site of the largest circumference between the waist and the thighs. The waist:hip ratio was calculated by dividing the waist circumference by the hip circumference.

**Body composition.** Total body water (TBW) was measured using the <sup>2</sup>H<sub>2</sub>O dilution technique (Schoeller *et al.* 1980; Van Marken Lichtenbelt *et al.* 1994). In the evening, the subjects collected a background urine sample and then ingested a dose of <sup>2</sup>H-enriched water (<sup>2</sup>H<sub>2</sub>O), after which they refrained from consuming fluid and food. The following morning, a urine sample from the second voiding was collected between 08.00 h and 10.00 h. The concentration of <sup>2</sup>H in the urine samples was measured using an isotope ratio mass spectrometer (Micromass Optima, Manchester, UK). TBW was obtained by dividing the measured <sup>2</sup>H dilution space by 1.04 (Schoeller *et al.* 1980). FFM was calculated by dividing the TBW by the constant hydration factor 0.73, which can be used for adult subjects (Schoeller, 1996). The weight loss and weight maintenance periods were long enough to establish a stable hydration of FFM. Fat mass (FM) was calculated by subtracting FFM from body weight. FM expressed as a percentage of total body mass is body fat percentage.

**Attitude towards eating.** To determine whether attitude towards food intake changed during the experiment, a Dutch translation of the TFEQ was used (Stunkard & Messick, 1985; Westtererp-Plantenga *et al.* 1999). The first factor of the TFEQ (F1) measures cognitive restrained eating: control of food intake by thought and will-power. The second factor (F2) represents disinhibition: an incidental inability to resist eating cues, or inhibition of dietary restraint, and emotional eating. The third factor (F3) examines the subjective feeling of general hunger.

In addition, the Herman–Polivy questionnaire was used to determine the frequency of dieting (Herman & Polivy, 1980).

**Post-absorptive appetite profile.** To determine the post-absorptive appetite profile, hunger and satiety were rated on anchored 100 mm visual analogue scales in the morning before breakfast.

**Blood parameters.** A fasting blood sample of 10 ml was taken and mixed with EDTA to prevent clotting. Plasma was obtained by centrifugation (at 3000 U/min for 10 min at 4°C), frozen in liquid nitrogen and stored at -80°C until further analysis. Plasma glucose concentrations were determined using the hexokinase method (Glucose HK 125 kit; ABX diagnostics, Montpellier, France). The Wako NEFA C-kit (Wako Chemicals, Neuss, Germany) was used to determine free fatty acid concentrations. Insulin concentrations were measured using the RIA-kit (Insulin RIA-100; Kabi-Pharmacia, Uppsala, Sweden). The glycerol kinase method was used to determine glycerol concentrations (Boehringer Mannheim GmbH, Mannheim,

**Table 1.** Subject characteristics of the protein (*n* 53) and the control (*n* 60) group at baseline, after 4 weeks on a very-low-energy diet (VLED; Modifast), and after 3 and 6 months weight maintenance

	Baseline				After 4 weeks VLED				After 3 months weight maintenance				After 6 months weight maintenance			
	Protein		Control		Protein		Control		Protein		Control		Protein		Control	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Body mass (kg)	83.1	11.1	83.4	10.4	76.7	9.9*	77.3	9.9*	76.7	11.0*	79.4	10.9*	77.5	11.8*†	80.3	11.6*
BMI (kg/m <sup>2</sup> )	29.3	2.5	29.4	2.6	27.0	2.3*	27.3	2.6*	27.0	2.6*	28.0	3.0*	27.3	2.9*†	28.3	3.2*
Waist (cm)	92.9	8.2	94.5	7.9	87.1	7.4*	88.9	7.1*	85.6	8.3*†	89.2	8.5*	85.8	9.6*††	89.5	9.2*
Waist:hip ratio	0.85	0.08	0.88	0.08	0.84	0.08	0.86	0.08*	0.83	0.07*	0.85	0.08*	0.83	0.08*	0.85	0.09*
Fat-free mass (kg)	51.3	9.7	52.1	9.1	49.0	9.0*	49.9	8.8*	50.5	9.8*	51.1	9.2*	50.6	9.4*	51.1	9.3*
Fat mass (kg)	31.0	6.4	31.4	5.9	27.0	6.2*	27.2	6.2*	25.5	6.3*†	28.2	6.7*	26.0	7.6*††	29.0	7.1*
% Body fat	37.8	6.1	37.8	5.9	35.6	6.7*	35.4	6.9*	33.7	6.7*	35.6	6.9*	33.8	7.4*†	36.2	7.2*
F1	8.6	3.8	7.7	3.8	10.4	4.5*	9.5	4.5*	11.5	4.4*	9.6	4.6*	11.4	4.1*	9.4	3.8*
F2	6.0	2.7	6.2	2.7	5.0	2.6*	5.3	2.6*	4.7	2.6*	5.5	2.6*	4.7	2.5*	5.6	2.8
F3	4.8	3.6	4.2	3.2	4.0	3.8	3.9	3.5	3.1	3.0*	3.4	3.0	3.1	3.5*	3.5	3.3
HP	15.8	3.7	15.6	3.6	16.7	3.6	16.7	3.8	17.2	3.5*	17.1	3.8*	17.0	3.6*	16.8	4.0*
Hunger (mmVAS)	43.8	26.8	33.6	25.4	37.8	25.4	34.2	24.3	27.2	20.1*	31.1	18.9	33.5	20.9	36.1	21.0
Satiety (mmVAS)	24.3	22.5	31.9	22.6	31.9	25.8	36.5	22.7	44.9	20.2*	38.1	21.2	36.3	25.6*†	38.0	23.2
% Regain									2.6	59.1†	37.9	45.7	19.6	82.1*†	54.9	65.8*
Rate of regain (g/d)									0.8	42.5†	22.9	30.9	4.7	31.0*†	17.1	20.0

F1, cognitive restraint; F2, disinhibition; F3, hunger (Three Factor Eating Questionnaire scores). HP, Herman-Polyvi restraint questionnaire score; hunger and satiety scores in the fasting state before breakfast, mmVAS, mm Visual Analogue Scale; % regain, body mass regain expressed as a percentage of body mass loss.  
 \* *P* < 0.05 compared with baseline (repeated measures ANOVA).  
 † *P* < 0.05 compared with control group (factorial ANOVA).  
 ‡ *P* < 0.05 interaction group × time (two-factor repeated measures ANOVA).

Germany). Triacylglycerol was measured using the GPO-trinder kit (Sigma Diagnostics Inc., St Louis, MO, USA). The  $\beta$ -hydroxybutyrate dehydrogenase method (Sigma Diagnostics Inc., St Louis, MO, USA) was used to determine  $\beta$ -hydroxybutyrate concentration. Leptin concentrations were measured using the human leptin RIA-kit (Linco Research Inc., St Charles, MO, USA).

**Adverse events.** Adverse events during treatment were recorded and the severity and outcome specified.

**REE and substrate oxidation.** In a random subset of subjects ( $n = 75$ ), REE and substrate oxidation were measured by means of an open-circuit ventilated hood system. REE was measured in the morning with subjects in a fasted state while lying supine for 30 min. Gas analyses were performed by a paramagnetic oxygen analyser (type 500A, Servomex, Crowborough, Sussex, UK) and an IR carbon dioxide analyser (type 500A, Servomex, Crowborough, Sussex, UK), similar to the analysis system described by Schoffelen *et al.* (1997). Calculation of REE was based upon the Weir's formulas (Weir, 1949). Respiratory quotient (RQ) was calculated as  $\text{CO}_2$  produced/ $\text{O}_2$  consumed.

**Physical activity.** The same subset of subjects who underwent metabolic testing was used to measure physical activity level (PAL). PAL was determined using a uniaxial accelerometer (CSA, CSAB, Inc. Stamford, CT, USA.) (Ekelund *et al.* 2000), or a tri-axial accelerometer for movement registration (Tracmor Maastricht University, Maastricht, The Netherlands.) (Goris *et al.* 2001), during 1 week. Subjects wore the CSA or Tracmor during waking hours on a belt at waist level on their back. The different accelerometers were randomised over the two groups. Half the subjects in the protein group and the control group received the CSA, the other half received the Tracmor. Subjects received the same accelerometer every time.

PAL was calculated using the following equations:

$$\begin{aligned} \text{CSA (Ekelund et al. 2000): PAL} \\ = (0.000001379 \times (\text{counts/day} \times 5)) + 1.113 \end{aligned}$$

$$\begin{aligned} \text{Tracmor (Goris et al. 2001): TEE} \\ = -1.259 + (1.552 \times \text{REE}) + (0.076 \times \text{counts/min}) \end{aligned}$$

$$\text{PAL} = \text{TEE}/\text{REE}$$

in which TEE is total energy expenditure (MJ/d) and REE is resting energy expenditure (MJ/d).

**Protein intake.** Compliance with additional protein intake was checked by taking 24 h urine samples after 3 months WM and analysing these for nitrogen. Energy intake from protein was calculated from the 24 h nitrogen output according to the formula of Isaksson (1980):

$$\begin{aligned} \text{Protein intake (g)} \\ = (\text{nitrogen output in 24 h urine (g/d)} + 2 \text{ g}) \times 6.25 \end{aligned}$$

**Energy intake and energy efficiency.** Energy intake was calculated as TEE plus energy storage (ES). Energy storage was calculated from the composition of the energy stored. A figure of 30 MJ per kilogram body weight gain (Pullar

& Webster, 1977) was taken for the usual energy storage of FM and FFM (A). If body weight gain consisted of only FFM while FM decreased, 52 MJ/kg FFM gain (Pullar & Webster, 1977) and 30 MJ/kg FM loss were used (B).

$$\text{(A) ES (MJ/d)} = (\Delta \text{ body weight (kg)} \times 30) / \text{number of days}$$

$$\text{(B) ES (MJ/d)} = ((\Delta \text{FFM (kg)} \times 52) - (\Delta \text{FM (kg)} \times 30)) / \text{number of days}$$

To see whether a higher percentage of energy ingested as protein in the diet could lower the energy efficiency, which is known already as the 'Stock' hypothesis (Dulloo & Jacquet, 1999; Stock, 1999), we used the following equation to calculate energy efficiency (Eeff):

$$\begin{aligned} \text{Eeff (kg/MJ)} = \text{body weight regain (kg)} / (\text{EI (MJ/d)} \\ \times \text{number of days}) \end{aligned}$$

#### VLED period

The VLED provided 2.1 MJ/d (carbohydrate-protein-fat 42:44:14 energy percentage) (Modifast, Novartis Nutrition, Breda, The Netherlands) and was supplied in three sachets daily that were dissolved in water to obtain a milk shake, pudding, soup or muesli. Vegetables and fruit were allowed in addition to Modifast. The aim was a body weight loss of at least 4 kg over 4 weeks.

#### WM period

After the VLED period, the WM period started, in which all subjects were allowed to eat their habitual diet again. During the WM phase, the subjects in the protein group received 30 g additional protein per day. This was provided as a sachet of pure protein (protein source calcium caseinate, 1.4% calcium) per day to be dissolved in water, giving rise to a vanilla-flavoured drink. The protein drink contained no carbohydrate or fat. Subjects were required to consume the protein drink at lunch or in the afternoon. In this way, we aimed at an energy intake comprising 18–20% protein/d, depending on the subject's usual protein intake.

#### Data analysis

Data are presented as mean and standard deviation (SD). A two-factor repeated measures ANOVA was carried out to determine possible differences between the protein and control groups in all measured parameters over time. When appropriate, a factorial ANOVA was used for analysing differences between the two groups. *Post hoc* analyses were made with the Scheffe F-test. A *P* value < 0.05 was regarded as statistically significant. Statistical procedures were performed using Statview SE + Graphics (Abacus Concepts, Berkeley, CA, USA).

#### Results

The effects did not differ between men and women so data from both genders were analysed together. No adverse events occurred. Subjects continuously reported that they

felt positive about taking part in the study. They confirmed that ample attention was given to their questions.

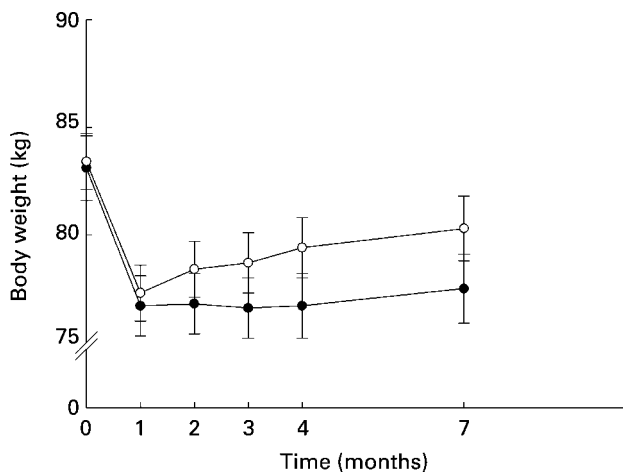
*VLED period*

During the VLED period, the changes described below occurred, which did not differ between the subsequent protein and control groups (Table 1). Subjects lost a significant amount of body weight, i.e. 6.3 (SD 2.0) kg, or 7.5 (SD 2.0) % of their original body weight ( $P < 0.0001$ ). The mean change in body weight for both groups over time is shown in Fig. 1. This consisted of 4.0 (SD 1.7) kg FM and 2.3 (SD 1.7) kg FFM. Waist circumference was also significantly reduced over time. Attitude towards eating showed some significant changes over time (Table 1). Cognitive restraint (F 1, TFEQ) increased significantly; disinhibition (F 2, TFEQ) and general hunger scores (F 3, TFEQ) decreased during weight loss (Table 1). REE and RQ decreased during weight loss (Table 2). TEE decreased in both groups, but this only reached significance in the control group. Fasting blood glucose, insulin, triacylglycerol and leptin levels showed a significant decrease with weight loss, and  $\beta$ -hydroxybutyrate, glycerol and free fatty acids showed a significant increase with weight loss (Table 3).

*WM period*

Compliance with the additional protein was shown by a higher amount of nitrogen in 24h urine in the protein group compared with the control group (14.3 (SD 3.5) g/d v. 11.2 (SD 3.5) g/d;  $P < 0.05$ ). The protein intake in the protein group was 101.7 (SD 22.2) g/d, which was significantly higher than the 82.7 (SD 22.0) g/d in the control group ( $P < 0.05$ ). When expressed as percentage of energy intake from protein, significant differences persisted, with 18 % in the protein group and 15 % in the control group ( $P < 0.05$ ).

During WM, percentage body weight regain, as well as rate of regain, was significantly lower in the protein group than the control group (Table 1; Fig. 2). The net percentage body mass lost after the WM period compared



**Fig. 1.** The change in body weight over time for the protein group (●) (*n* 53) and the control group (○) (*n* 60). Values are means with standard deviations of the mean shown by vertical bars.

**Table 2.** Energy expenditure and substrate oxidation of the protein (*n* 32) and the control (*n* 43) group at baseline, after 4 weeks on a very-low-energy diet (VLED; Modifast) and after 3 and 6 months weight maintenance

	Baseline		After 4 weeks VLED		After 3 months weight maintenance		After 6 months weight maintenance	
	Protein		Control		Protein		Control	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Resting energy expenditure (MJ/d)	6.80	1.1	6.74	1.0	6.10	0.8*	6.70	1.1
Respiratory quotient	0.84	0.04	0.83	0.05	0.78	0.04*	0.82	0.06
Physical activity level	1.7	0.04	1.6	0.2	1.6	0.2	1.7	0.2
Total energy expenditure (MJ/d)	11.2	1.3	10.7	1.9	9.7	1.3*	10.4	1.7

\*  $P < 0.05$  compared with baseline (repeated measures ANOVA).  
 †  $P < 0.05$  compared with control group (factorial ANOVA).

**Table 3.** Fasting blood-parameters of the protein (*n* 32) and the control (*n* 43) group at baseline, after 4 weeks on a very-low-energy diet (VLED; Modifast) and after 3 months weight maintenance

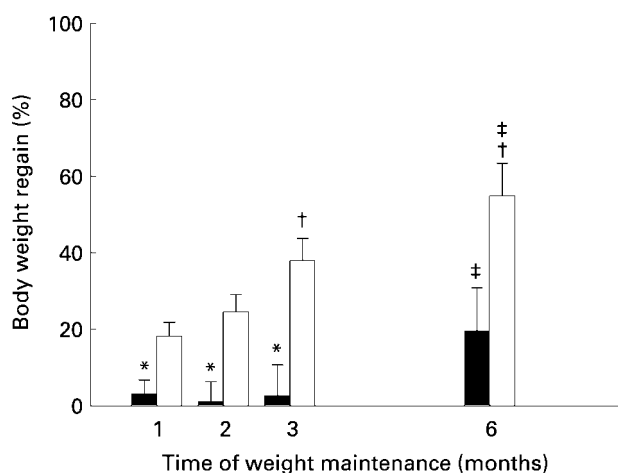
	Baseline				After 4 weeks VLED				After 3 months weight maintenance			
	Protein		Control		Protein		Control		Protein		Control	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Glucose (mmol/l)	5.5	0.5	5.6	1.0	5.2	0.4*	5.2	0.6*	5.4	0.5	5.3	0.6*
Insulin ( $\mu$ U/ml)	10.8	5.3	9.9	3.5	6.7	2.4*	7.0	3.3*	7.9	6.0*	8.4	3.9*
$\beta$ -Hydroxy butyrate ( $\mu$ mol/l)	233.0	70.0	248.8	87.9	537.0	272.9*	483.6	348.5*	256.4	87.3	255.2	89.4
Glycerol ( $\mu$ mol/l)	93.3	38.1	94.1	35.2	109.6	53.0*	104.3	69.7*	90.7	36.2	86.5	34.3
Free fatty acids ( $\mu$ mol/l)	300.6	122.1	323.6	145.5	441.1	188.5*	402.4	226.8*	297.6	151.7	273.8	134.2
Triacylglycerol ( $\mu$ mol/l)	1.2	0.6	1.6	0.9	0.9	0.3*	1.0	0.5*	1.0	0.5	1.3	0.7
Leptin ( $\mu$ g/l)	21.7	10.7	22.7	11.8	8.7	5.7*	10.7	7.1*	15.8	10.4*†‡	20.4	11.9

\*  $P < 0.05$  compared with baseline (repeated measures ANOVA).

†  $P < 0.05$  compared with control group (factorial ANOVA).

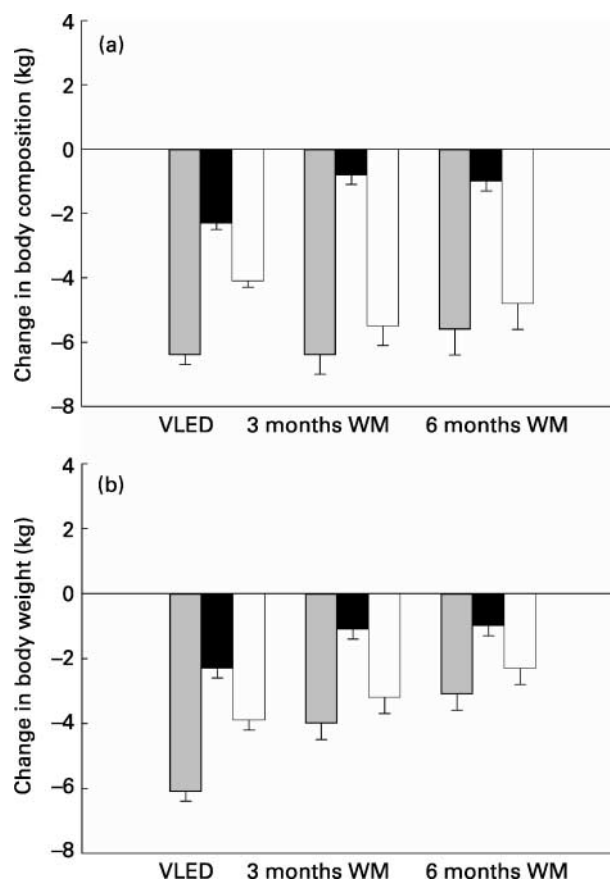
‡  $P < 0.05$  interaction treatment  $\times$  time (two-factor repeated measures ANOVA).

with baseline was significantly higher in the protein group than the control group (6.7 (SD 7.2) % *v.* 3.8 (SD 4.8) %;  $P < 0.05$ ). The protein group showed a decrease in waist circumference during WM, whereas the control group showed an increase ( $-1.2$  (SD 0.7) cm *v.* 0.5 (SD 0.5) cm;  $P < 0.05$ ). FFM stayed significantly lower during WM compared with baseline in both groups. FM in the protein group continued to decrease during WM, while FM increased in the control group ( $-0.9$  (SD 0.7) kg *v.* 1.7 (SD 0.4) kg;  $P < 0.005$ ) (Fig. 3). Cognitive restraint scores stayed significantly higher during WM compared with baseline in both groups. No differences were seen in the change in cognitive restraint during the study between the groups. Disinhibition stayed significantly lower in both groups, and general hunger decreased in both groups. Satiety in the fasted state before breakfast increased significantly during WM in the protein group but not in the control group (Table 1). The increase in satiety was significantly higher in the protein group than the control group. The hunger scores in the fasted state before breakfast did not change over time in either group.



**Fig. 2.** The weight regain during weight maintenance (expressed as percentage of the weight loss) for the protein (■) (*n* 53) and the control (□) (*n* 60) groups. Values are means with standard deviations of the mean shown by vertical bars. \* $P < 0.05$  compared with control group, † $P < 0.05$  compared with 1 month weight maintenance, ‡ $P < 0.05$  compared with 2 months weight maintenance.

REE returned to almost baseline values in both groups during WM (Table 2). To assess possible differences in REE adjusted for FFM between groups, we analysed the residuals of the regression of REE *v.* FFM. The residual analysis showed no significant group  $\times$  time interaction. The increase in RQ during WM was significantly smaller



**Fig. 3.** The changes in body weight (■), fat-free mass (■) and fat mass (□) for (a) the protein group (*n* 53) and (b) the control group (*n* 60) during the study compared with baseline. Values are means with standard deviation of the mean shown by vertical bars. VLED, change after the very-low-energy diet period; 3 months WM, change after 3 months weight maintenance; 6 months WM, change after 6 months weight maintenance.

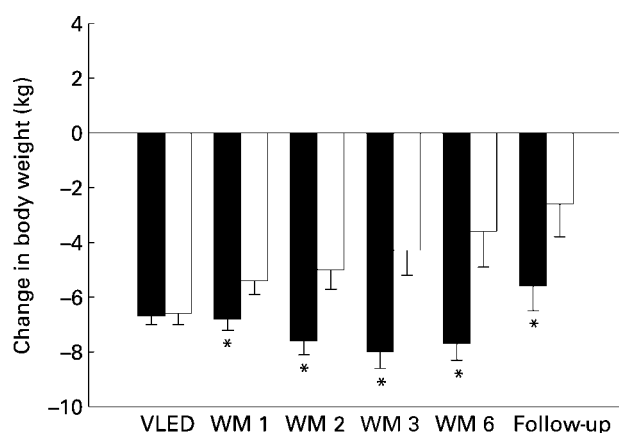
in the protein group than the control group (0.03 (SD 0.01) v. 0.07 (SD 0.01);  $P < 0.05$ ), indicating a smaller decrease in fat oxidation in the protein group than in the control group. The increase in RQ was not related to weight regain ( $P > 0.05$ ). No changes in PAL were seen over time and between groups (Table 2). A trend for a lower energy efficiency of the protein group than the control group was found after 6 months WM ( $3.0 \times 10^{-4}$  (SD 0.003) kg/MJ v. 0.001 (SD 0.002) kg/MJ;  $P = 0.12$ ).

Leptin concentrations stayed significantly lower after treatment compared with baseline in the protein group (Table 3). Glucose concentrations stayed significantly lower after treatment compared with baseline in the control group. Insulin concentrations stayed significantly lower after treatment compared with baseline in both groups.  $\beta$ -Hydroxybutyrate, glycerol and free fatty acid concentrations returned almost to baseline values during weight regain (Table 3). Triacylglycerol concentrations during WM were significantly lower in the protein group than the control group.

The change in body weight of the subset of subjects (protein  $n = 31$ , control  $n = 39$ ) who were also measured after 6 months follow-up is shown in Fig. 4. The analysis of the follow-up measurement of body weight showed a significant group  $\times$  time interaction.

## Discussion

In the present study, we investigated whether the addition of protein to the diet might limit weight regain after a weight loss of 5–10% in moderately overweight men and women. The results show that subjects who consumed 18% of their energy intake as protein regained less weight during 6 months WM (0.8 kg), compared with subjects who consumed 15% of their energy intake as protein (3.0 kg). This was independent of changes in cognitive restraint, physical activity, REE, TEE and hunger scores since these parameters did not differ between groups.



**Fig. 4.** The change in body weight for the subjects with follow-up in the protein group (■) ( $n = 31$ ) and the control group (□) ( $n = 39$ ) during the study compared with baseline. Values are means with standard deviations of the mean shown by vertical bars. \* $P < 0.05$  compared with the control group. VLED, change after the very-low-energy diet period; WM 1, 2, 3, 6 change after 1, 2, 3 and 6 months weight maintenance.

The composition of the body mass regained was different for the protein and the control groups. The body mass regained in the protein group consisted only of FFM, whereas the FM still decreased during WM, which resulted in a lower percentage of body fat. In the control group, the composition of the body mass regain was FM as well as FFM. The observation that body mass regain consisting only of FFM results in a slower weight regain is in line with our hypothesis that when the composition of the weight regained consists of a greater FFM, the inevitable increase in body mass will be slower. Here, however, we have shown that this was due to increased protein intake without a change in physical activity, whereas in the study of Pasman *et al.* (1999), the cause appeared to be increased physical activity.

In addition to a beneficial effect of dietary protein during weight loss (Skov *et al.* 1999; Astrup, 2001; Dumesnil *et al.* 2001; Layman *et al.* 2003), protein also appears to support WM.

Although subjects were asked to consume 30 g/d protein in addition to their usual diet, the analysis of the results showed a mean difference of 19 g/d between the protein and the control group. This could imply that subjects in the protein group replaced part of their usual protein intake by the protein that was provided to them. Since this was a study under free-living conditions, the carbohydrate and fat content of the weight maintenance diet remains unknown.

With respect to satiety, we found a greater increase in post-absorptive satiety in the protein group during WM, although their energy intake did not differ significantly from that of the control group. Short-term differences in digestion, absorption and energy expenditure between different protein sources have been shown (Boirie *et al.* 1997; Mikkelsen *et al.* 2000). Boirie *et al.* (1997) introduced the concept of 'fast' and 'slow' proteins, based upon the differences in digestion and absorption of these proteins. These short-term effects may be related to increases in the concentrations of gut hormones, such as glucagon-like peptide-1 (Flint *et al.* 1998; Gutzwiller *et al.* 1999; Naslund *et al.* 1999) and cholecystokinin (Schick *et al.* 1991; Burton-Freeman *et al.* 2002; Kissileff *et al.* 2003). However, this probably does not apply to post-absorptive satiety. Animal protein has been shown to introduce a higher energy expenditure (Mikkelsen *et al.* 2000), and, in the longer term, the higher post-absorptive satiety and thermogenesis were sustained with high-protein diets consisting of a variety of proteins from different sources (Dulloo & Jacquet, 1999; Westerterp-Plantenga *et al.* 1999; Dumesnil *et al.* 2001; Westerterp-Plantenga *et al.* 2004). Moreover, a relationship between satiety and thermogenesis has been shown (Crovetti *et al.* 1998; Westerterp-Plantenga *et al.* 1999) with using high-protein diets. We therefore suggest that the higher post-absorptive satiety is due to increased thermogenesis.

The observation that the increase in REE as a function of FFM during WM did not differ significantly might be due to the lack of a significant difference in the increase of FFM.

The slower increase in RQ in the protein group reflects a more favourable body composition.

Although the treatment with respect to number of visits, measurements and attention was identical in both groups,

there was no placebo used for the additional protein, similar to previous meal-replacement studies (Ditschuneit *et al.* 1999; Flechtner-Mors *et al.* 2000; Rothacker, 2000; Ashley *et al.* 2001; Allison *et al.* 2003). The issue of the placebo is a difficult one. It would be possible to exchange the protein drink for a carbohydrate or fat drink, but then one needs a more complete study that also addresses the specific carbohydrate and fat effect. This was beyond the scope of the present study. However, we checked whether the results in both groups could be differently related to changes in eating behaviour. The increase in cognitive restraint during the study did not differ between the protein and control group so this cannot explain the limited body weight regain during WM in the protein group compared with the control group. It shows that, without a difference in increase in cognitive restraint, the protein group maintained the weight loss better.

Since reporting of food intake in humans cannot be expected to be completely reliable because of reasons of privacy (Blundell, 2000), and thus often leads to incorrect conclusions (Goris & Westerterp, 1999, 2000; Goris *et al.* 2000), we chose to calculate energy intake from energy expenditure and energy storage. The calculated energy intake was not significantly different between both groups and therefore cannot explain the difference in body weight regain.

Zemel (2003) showed that a low calcium intake (400 mg/d) leads to a smaller body weight loss compared with a high calcium intake. Since the average calcium intake in the Dutch population is already rather high (800–1600 mg/d; Hulshof *et al.* 2003), the additional calcium intake in the protein group (420 mg/d) could have contributed to the difference in body weight regain in the present study, yet is unlikely to explain it completely.

The analysis of the follow-up measurement of body weight showed a significant difference between both groups after 3 and 6 months WM. The change in body weight over time in the control group appeared to continue as during the first 6 months, whereas the body weight over time in the protein group appeared to be maintained. Unfortunately, body composition was not determined during follow-up. This needs to be repeated in order to be able to indicate whether body composition is still a main factor in determining the difference in WM between the groups. With respect to metabolic syndrome risk factors, WM as well as waist circumference was more favourable in the protein group than in the control group.

In conclusion, a protein intake of 18% *v.* 15% resulted in improved WM over 6–12 months in moderately overweight subjects after a weight loss of 7.5%. This improved WM implied several factors, *i.e.* improved body composition, fat distribution, substrate oxidation and satiety. The result suggests that improved WM is possible when it is supported multi-factorially.

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